

Studies on the Constituents of *Chloranthus* spp. I. The Structures of Two New Amides from *Chloranthus serratus* and the Isolation of Isofraxidin from *C. japonicus*

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Two kinds of new amides were isolated from *Chloranthus serratus* and characterized as N- β -phenethyl-3-(3,4-methylenedioxyphenyl)propenamide (I) and N- β -phenethyl-3-(3,4-dimethoxyphenyl)propenamide (II). Isofraxidin (III) was identified as a constituent of *C. japonicus*.

Five species of *Chloranthus* genus (Chloranthaceae) are known to grow in Japan.²⁾ Among them *C. serratus* (THUNB.) ROEM. et SCHULT. and *C. japonicus* SIEB. (Japanese name: Hutarishizuka and Hitorishizuka, respectively) grow in China too, and the dried roots are expected useful as an antifungal drug in Chinese medicine.³⁾

Very few are the constituents of the species of this genus studied except (—)- α -copaene which has been isolated from the *Chloranthus* oil of Chinese origin (*C. spicatus* (THUNB.) MAKINO ?) for the structural elucidation research.⁴⁾ Then, we started our studies on the constituents of the plants of *Chloranthus* genus, intending to clarify the constituents and looking for the antifungally potent compounds. Here in the course of our research, we wish to report about the isolation of two new amides (I and II) from *C. serratus* along with the evidences for the structural elucidation as N- β -phenethyl-3-(3,4-methylenedioxyphenyl)propenamide and N- β -phenethyl-3-(3,4-dimethoxyphenyl)propenamide. Also, we want to mention briefly about the identification of isofraxidin (III) as a constituent of *C. japonicus*.

The amides (I and II) were obtained through silica gel chromatography of the ethyl acetate extract prepared from the methanol extract of the aerial part of *C. serratus*. On the other hand, isofraxidin (III) was isolated through silica gel chromatography of the ethyl acetate extract prepared from the methanol extract of the roots of *C. japonicus*. The properties of I—III are summarized in Table I, Fig. 1 and Fig. 2. These properties, especially the spectroscopical ones, suggested N- β -phenethyl-3-(3,4-methylenedioxyphenyl)propenamide

TABLE I. Properties of the Isolated Compounds (I—III)

	mp (°C)	mol. formula	UV $\lambda_{\text{max}}^{\text{MeOH}}$ $m\mu$ (log ϵ)	IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1}
I	135	$\text{C}_{18}\text{H}_{17}\text{O}_3\text{N}$	321(4.60), 289(4.53), 282(4.50)	3300, 1658, 1615, 1542, 1500
II	138	$\text{C}_{19}\text{H}_{21}\text{O}_3\text{N}$	315(4.65), 289(4.62), 282(4.58)	3330, 1650, 1620, 1600, 1540, 1512
III	148.5—150	$\text{C}_{11}\text{H}_{10}\text{O}_5$	212(4.34), 228(3.95), 344(3.81)	3340, 1700, 1580, 1290

1) Location: Aobayama, Sendai.

2) J. Ohwi, "Flora of Japan," Shibundo, Tokyo, 1969, p. 462.

3) "Iconographia Cormophytorum Sinicorum," Tomus I, Chinese Academic Press, Peking, 1972, p. 347.

4) P. de Mayo, R.E. Williams, G. Büchi and S.H. Fearheller, *Tetrahedron*, **21**, 619 (1965).

for I; N- β -phenethyl-3-(3,4-dimethoxyphenyl)propenamide for II and isofraxidin for III. These suggestion was certified by a direct comparison with the synthesized amides (I and II) and the authentic sample of isofraxidin.

Piperonal and malonic acid were refluxed in pyridine to produce 3-(3,4-methylenedioxyphenyl)propenoic acid (IV), which was methylated with methanolic hydrogen chloride. The methyl ester (V) was heated with β -phenethylamine to afford the amide, identified to the natural amide (I).

3,4-O-Dimethylcaffeic acid methyl ester (VI) which was obtained in the result of methylation of ferulic acid using with dimethyl sulfate, was heated in β -phenethylamine to give the amide, identical in all aspects to the isolated amide (II).

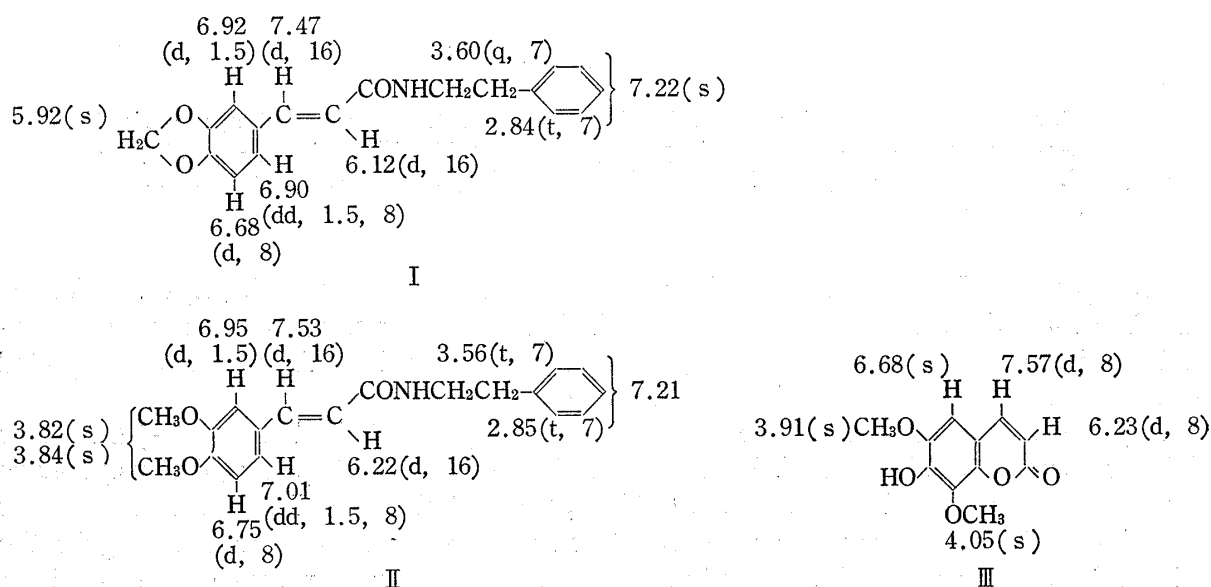


Fig. 1. NMR Spectra's Assignment of I, II, and III (in CDCl_3)

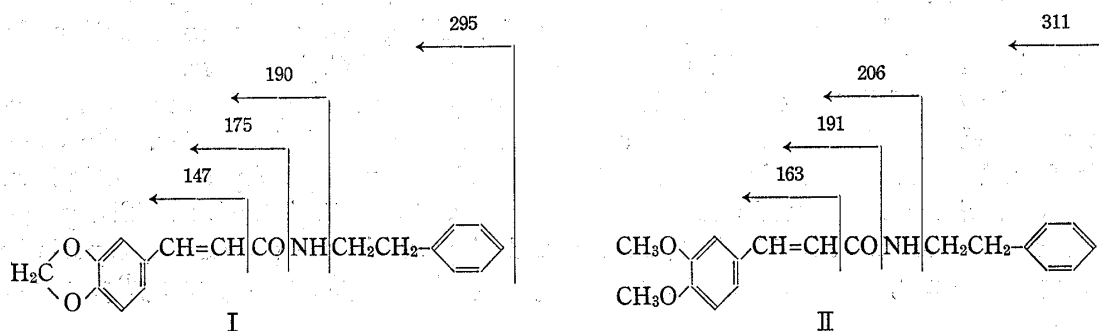


Fig. 2. Mass Spectra's Data of I and II

Experimental⁵⁾

Isolation Procedure of Amides (I and II)—The dried aerial parts (6.8 kg) of *C. serratus*, which was collected in the mountainous area in the west of Sendai city in June, were extracted with hot MeOH. The solvent was evaporated to give 2 liters of the extract, which was extracted with AcOEt again. The latter extract (150 g) was chromatographed on silica gel (1.3 kg) to fractionate many eluates, the benzene-AcOEt (10:1) eluate of which afforded I (1.2 g) and the benzene-AcOEt (3:1) eluate provided II (0.5 g). I was obtained as colorless needles after recrystallization from acetone-*n*-hexane mixture, having the following properties: mp 135°. *Anal.* Calcd. for $\text{C}_{18}\text{H}_{17}\text{O}_3\text{N}$: C, 73.20; H, 5.80; N, 4.74. Found: C, 72.94; H, 5.97;

5) Melting points were determined on a hot stage of Yazawa Apparatus and uncorrected. Chemical shifts were reported as ppm on scale using tetramethylsilane as internal reference, except a specific case (see footnote 6).

N, 4.63. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3300, 1658, 1615, 1542, 1500, 1440, 1247. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 321 (4.61), 289 (4.53), 282 (4.50). NMR (CDCl_3) δ : 2.84 ppm (2H, t, $J=7$ Hz), 3.60 (2H, q, $J=7$), 5.92 (2H, s), 6.12 (1H, d, $J=16$), 6.68 (1H, d, $J=8$), 6.90 (1H, dd, $J_1=8$, $J_2=1.5$), 6.92 (1H, d, $J=1.5$), 7.22 (5H, s), 7.47 (1H, d, $J=16$). NMR (CD_3OD) δ : 2.83 (2H, t, $J=7$), 3.52 (2H, t, $J=7$), 5.84 (2H, s), 6.29 (1H, d, $J=16$), 6.72 (1H, d, $J=8$), 6.94 (1H, dd, $J_1=8$, $J_2=1.5$), 6.99 (1H, d, $J=1.5$), 7.20 (5H, s), 7.37 (1H, d, $J=16$). Mass Spectrum m/e (relative abundance): M^+ , 295 (23), 190 (34), 175 (100), 147 (25), 145 (19), 117 (14), 89 (24), 63 (11). II was obtained as colorless needles after recrystallization from acetone-*n*-hexane mixture, characterized as follows: mp 138°. Anal. Calcd. $\text{C}_{19}\text{H}_{21}\text{O}_3\text{N}$: C, 73.29; H, 6.80; N, 4.50. Found: C, 73.34; H, 7.02; N, 4.53. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3330, 1650, 1620, 1600, 1540, 1512. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 315 (4.65), 289 (4.62), 282 (4.58). NMR (CDCl_3) δ : 2.85 (2H, t, $J=7$), 3.56 (2H, t, $J=7$), 3.82 (3H, s), 3.84 (3H, s), 6.22 (1H, d, $J=16$), 6.75 (1H, d, $J=8$), 6.95 (1H, d, $J=1.5$), 7.01 (1H, dd, $J_1=8$, $J_2=1.5$), 7.21 (5H, s), 7.53 (1H, d, $J=16$). Mass Spectrum m/e (relative abundance): M^+ , 311 (21), 206 (30), 191 (100), 163 (12), 91 (11), 77 (12), 65 (5).

Isolation and Identification of Isofraxidin (III)—The dried roots (1.3 kg) of *C. japonicus*, which was collected in the mountainous area in the northwest of Sendai city in May, were extracted with hot MeOH. The solution was concentrated to 1 liter of the extract, which was extracted with AcOEt again. The final extract (80 g) was chromatographed on silica gel (1.0 kg) to fractionate many eluates, the benzene-AcOEt (20:3) eluate among which gave colorless needles (III, 90 mg), after recrystallization from AcOEt, mp 148.5–150°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3440, 1700, 1580, 1290. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 212 (4.34), 228 (3.95), 344 (3.81). Mass Spectrum m/e (relative abundance): M^+ , 222 (100), 207 (28), 194 (14), 179 (17), 151 (10), 123 (12). NMR (CDCl_3) δ : 3.91 (3H, s), 4.05 (3H, s), 6.23 (1H, d, $J=8$), 6.63 (1H, s), 7.57 (1H, d, $J=8$). This compound was identified to isofraxidin, comparing with the authentic sample.

3-(3,4-Methylenedioxyphenyl)propenoic Acid (IV)—Piperonal (1.5 g) and malonic acid (4.2 g) were refluxed in pyridine (20 ml) for 24 hr. After concentrating the reaction solution in reduced pressure, sodium hydroxide solution was added to the residue to make an alkaline solution, from which the ether soluble fraction was removed. The alkaline solution was acidified with dil. HCl and extracted with AcOEt. The extract was washed with water, dried over anhydrous sodium sulfate. The residue after the removal of drying reagent and the solvent, provided colorless needles after recrystallization from AcOEt, mp 240°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3100–2500, 1690, 1627, 1605, 1495. NMR (D_2O -NaOD) δ : 5.92 (2H, s), 6.25 (1H, d, $J=16$), 6.75 (1H, d, $J=8$), 6.97 (1H, dd, $J_1=8$, $J_2=1.5$), 6.98 (1H, d, $J=1.5$), 7.23 (1H, d, $J=16$).

3-(3,4-Methylenedioxyphenyl)propenoic Acid Methyl Ester (V)—A suspension of IV (1 g) in MeOH (20 ml) was saturated with dried HCl gas and stirred for 1 hr. The reaction mixture was concentrated *in vacuo*, the residue recrystallized from MeOH to give amorphous (0.8 g) of V, mp 138°. Anal. Calcd. for $\text{C}_{11}\text{H}_{10}\text{O}_4$: C, 64.07, H, 4.89. Found: C, 64.33; H, 4.91. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1700, 1620, 1598, 1490, 1450. NMR (CDCl_3) δ : 3.77 (3H, s), 5.97 (2H, s), 6.21 (1H, d, $J=16$), 6.74 (1H, d, $J=8$), 6.99 (1H, d, $J=1.5$), 7.00 (1H, dd, $J_1=8$, $J_2=1.5$), 7.57 (1H, d, $J=16$).

Synthesis of I—V (0.7 g) was added to excess of β -phenethylamine and the mixture was refluxed for 1 hr. After cooling, the products were extracted with AcOEt, the AcOEt layer washed with dil. HCl, and then water, dried over anhydrous sodium sulfate. The drying reagent and the solvent were removed, the residue (0.8 g) chromatographed on silica gel. The benzene eluate gave the major product (0.3 g), which was identified to the naturally isolated amide (I), by mixed melting point test and comparison of IR and NMR spectra.

3,4-O-Dimethylcaffeic Acid Methyl Ester (VI)—Ferulic acid (100 mg) was dissolved to acetone (10 ml) and potassium carbonate (300 mg) was added to the solution, followed by the addition of dimethyl sulfate, refluxed for 1 hr. Water was added to the reaction mixture and the mixture was extracted with AcOEt. The ethyl acetate layer was washed with water, dried over Na_2SO_4 . The residue after the removal of the drying reagent and the solvent was recrystallized from acetone-*n*-hexane mixture to precipitate colorless prisms (90 mg, VI), mp 70–71°. IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 1690, 1620, 1595, 1508, 1249. NMR (CDCl_3) δ : 3.79 (3H, s), 3.89 (6H, s), 6.28 (1H, d, $J=16$), 6.83 (1H, d, $J=8$), 7.03 (1H, d, $J=1.5$), 7.11 (1H, dd, $J_1=8$, $J_2=1.5$), 7.63 (1H, d, $J=16$).

Synthesis of II—VI (90 mg) was dissolved to excess of β -phenethylamine and the solution was refluxed for 1 hr. After the same treatment described in the synthesis of I, the major product (60 mg) was identified to the naturally occurring amide (II) by mixed melting point test and comparison of IR and NMR spectra.

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